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(54) Title: PREVENTION OR TREATMENT OF SEPSIS WITH DANTROLENE OR AZUMOLENE

(57) Abstract

A method of preventing and treating sepsis in a human or other mammal afflicted with sepsis comprising the administration of a safe and effective amount of a compound that inhibits the release of calcium from the sarcoplasmic and/or endoplasmic reticula, preferably dantrolene sodium or azumolene sodium.

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# PREVENTION OR TREATMENT OF SEPSIS WITH DANTROLENE OR AZUMOLENE.

This invention relates to methods of preventing and treating sepsis in humans or other mammals. In particular, this invention relates to methods of preventing and treating sepsis comprising the administration of compounds that inhibit the release of calcium from the sarcoplasmic and endoplasmic reticula, preferably dantrolene sodium and/or azumolene sodium.

Sepsis is the leading cause of death in surgical intensive care units and neonatal units. In the United States alone, four hundred thousand (400,000) patients per year develop sepsis. Approximately, one hundred thousand (100,000) of these patients ultimately expire. The plethora of articles discussing the gravity of the sepsis problem is indicative of the need for more effective management of sepsis. *See*, e.g. Glauser, M. P., et al., "Septic Shock: pathogenesis", 338 *Lancet* 732 (September 21, 1991); Fein, A. M., et al., "Sepsis Syndrome", 10(11) *Infectious Disease Newsletter* 89-96 (1991); Parillo, J. E., "Management of Septic Shock: Present and Future", 115(6) *Ann. Intern. Med.* 491-493 (September 15, 1991); DiPiro, J. T., "Pathophysiology and Treatment of gram-negative sepsis", 47(3) *American Journal of Hospital Pharmacy* S6-S10 (November 1990); Bone, R. C., "The Pathogenesis of Sepsis", 115(6) *Ann. Intern. Med.* 457-469 (September 15, 1991); Dudley, M. N., "Overview of Gram Negative Sepsis", 47(3) *American Journal of Hospital Pharmacy* S3-S6 (November 1990); Barriere, S., et al., "Gram-negative sepsis, the sepsis syndrome, and the role of antiendotoxin monoclonal antibodies", 11(3) *Clin. Pharm.* 223-235 (March 1992) and Murray, M. J., et al., "Sepsis and septic shock - Deadly complications that are on the rise", 90(1) *Postgraduate Medicine* 199-202, 205-6, 208 (July 1991).

Surgical Intensive Care and trauma patients are those most likely to be afflicted with sepsis. Sepsis is a clinical syndrome characterized by leukocyte proliferation, altered mental state, tachypnea, tachycardia, metabolic acidosis, impaired renal and hepatic function, hypotension and hyperthermia or hypothermia. Sepsis results from infection with gram-positive or gram-negative bacteria, pathogenic viruses, fungi or rickettsia or the by-products of such infectious agents. If untreated, sepsis can progress to septic shock, wherein the patient manifests all the symptoms of sepsis plus hypotension (the systolic blood pressure drops below 90 mmHg or it decreases below the baseline systolic blood pressure by 40 mmHg).

At the cellular level, there is evidence that sepsis causes an increase in intracellular calcium. The importance of maintaining intracellular calcium homeostasis is described in Rasmussen, H., et al., "Calcium Ion as Intracellular Messenger and Cellular Toxin", 84 *Environmental Health Perspective* 17-25 (1990); Pounds, J. G., "The Role of Cell Calcium in Current Approaches to Toxicology", 84 *Environmental Health Perspectives* 7-15 (1990); Farber, J. L., "The Role of Calcium Ions in Toxic Cell Injury", 84 *Environmental Health Perspectives* 107-111 (1990); and Zaloga, G. P., et al. "Low Dose Calcium Administration Increases Mortality During Septic Peritonitis in Rats", 37 *Circul. Shock* 226-229 (1992).

If cellular calcium homeostasis is not maintained, as in sepsis, then, the elevation in intracellular calcium ion concentration causes activation of enzymes which 1) hydrolyze the cell membrane, and 2) cleave the cytoskeletal backbone of the cell resulting in a loss of cellular organization, and 3) cause fragmentation of the cellular DNA which controls protein synthesis. Disruption of the cellular organization eventually leads to tissue and organ destruction. *See*, Benson, D. W., et al., "Effect of sepsis on calcium uptake and content in skeletal muscle and regulation in vitro by calcium of total and myofibrillar protein breakdown in control and septic muscle:

Results from a preliminary study", 106(1) *Surgery* 87-93 (July 1989).

Current treatment of sepsis includes antimicrobial therapy, and respiratory and hemodynamic support. Yet, in spite of antimicrobial therapy, many patients still expire. Moreover, post-mortem examination of these patients reveals no signs of microbial infection, indicating that the downward course may continue in the absence of continued infection. Given the limited utility of supportive and antimicrobial therapy in treating sepsis, research has been conducted with compounds that affect intracellular calcium homeostasis.

Experimentally, it has been shown that the voltage sensitive calcium channel blockers, such as verapamil (an anti-hypertensive), increase the survival rate of Escherichia coli (*E. coli*) induced sepsis in dogs and mice. It is postulated that the calcium channel blockers act by decreasing calcium entry from the outside of the cell into the interior of the cell. Thus, increased intracellular calcium due to the influx of extracellular calcium is prevented by verapamil treatment, and, in those cells where verapamil acts effectively, it may diminish some of the deleterious effects of intracellular calcium overload. *See*, Bosson, S., et al., "Increased Survival with Calcium Antagonists in Antibiotic-Treated Bacteremia", 19 *Circulatory Shock* 69-74 (1986); Bosson, S., et al., "Verapamil Improves Cardiac Function and Increases Survival in Canine E. coli Endotoxin Shock", 16 *Circulatory Shock* 307-316 (1985); Lee, H., et al., "Protective Action of Calcium Entry Blockers in Endotoxin Shock", 18 *Circulatory Shock* 193-203 (1986).

However, it is theorized that, merely blocking the entry of calcium into the cell, as does verapamil, does not prevent the release of intracellular calcium from the sarcoplasmic and endoplasmic reticula. Thus, it is postulated that, in the case of sepsis, the calcium released from the sarcoplasmic and endoplasmic reticula, and the sustained levels of intracellular calcium, activate the enzyme cascade which leads to cellular destruction. The muscle relaxants, dantrolene sodium and

azumolene sodium, are compounds known to inhibit the release of intracellular calcium from the sarcoplasmic reticulum. *See*, Physicians Desk Reference, 46th Edition (1992).

In vitro research utilizing a cultured cell line has shown that dantrolene sodium inhibits the elevation of intracellular free calcium caused by enteropathogenic *Escherichia coli* (E. coli) which is the causative agent for infantile diarrhea. The investigators hypothesized that an increase in intracellular calcium leads to protein depolymerization and an eventual loss of absorptive capacity by the intestinal villi. *See*, Baldwin, T. J., et al., "Elevation of Intracellular Free Calcium Levels in HEp-2 Cells Infected with Enteropathogenic *Escherichia coli* (E. coli)", 59(5) *Infection and Immunity* 1599-1604 (May 1991).

Conversely, other research indicates that dantrolene did not significantly affect total or myofibrillar protein breakdown in rat septic muscle during sepsis. *See*, Benson, D. W., et al., "Effect of sepsis on calcium uptake and content in skeletal muscle and regulation in vitro by calcium of total and myofibrillar protein breakdown in control and septic muscle: Results from a preliminary study", 106(1)*Surgery* 87-93 (July 1989). Most recently, a study was made to assess the potential danger of using dantrolene sodium when a misdiagnosis of malignant hyperthermia was made in cases of sepsis. The symptoms of sepsis closely resemble those exhibited by patients afflicted with malignant hyperthermia. The study, done on rats and dogs, showed that dantrolene sodium did not adversely affect animals with sepsis, and the investigators concluded that the drug was probably safe to use even if the diagnosis of malignant hyperthermia versus sepsis was not entirely clear. In these experiments, no assessment of the utility of dantrolene sodium in treating sepsis was made. *See*, Beebe, D. S., et al., "Is Dantrolene Safe to Administer in Sepsis", 73 *Anesth. Analg.* 289-294 (1991).

Clearly, the use of dantrolene sodium and azumolene sodium to treat sepsis is undisclosed in the art. Nor does the art suggest that dantrolene sodium and azumolene sodium are useful

for sepsis treatment. Furthermore, the use of dantrolene sodium and azumolene sodium to treat sepsis is superior to the proposed use of verapamil which also affects cellular calcium. Verapamil exerts its effects on the voltage sensitive calcium channels which predominate in smooth and skeletal and cardiac muscle cells. *See*, Goodman and Gilman, The Pharmacological Basis of Therapeutics, Chap. 32 (8th Edition 1990). Dantrolene sodium and azumolene sodium modulate, directly or indirectly, calcium regulation by the sarcoplasmic and/or endoplasmic reticula, which are intracellular organelles, where calcium is stored, found in abundance not only in cardiac muscle cells, smooth muscle cells, and skeletal muscle cells, but also in brain cells, liver cells, pancreas cells and osteoblasts. Thus, compounds such as dantrolene sodium and azumolene sodium can exert their effects not only in muscle tissue, but on other major organ systems that may be targets of damage in sepsis. The use of dantrolene sodium and azumolene sodium to treat sepsis is clearly an advance in the art of sepsis management.

## SUMMARY OF THE INVENTION

A method of preventing and treating sepsis in a human or other mammal afflicted with sepsis comprising the administration of a safe and effective amount of a compound that inhibits the release of calcium from the sarcoplasmic and/or endoplasmic reticula, preferably dantrolene sodium and azumolene sodium.

## DEFINITIONS AND USAGE OF TERMS

The following is a list of definitions for the terms used herein:

The term "tachypnea", as used herein, means very rapid breathing usually at a rate of greater than twenty (20) breaths per minute.

The term "tachycardia", as used herein, means very rapid beating of the heart, usually at a rate of greater than ninety (90) beats per minute.

The term "sarcoplasmic reticulum" (plural - sarcoplasmic reticula), as used herein, means the intracellular organelle

where calcium is stored. The sarcoplasmic reticulum predominates in cardiac, smooth, and skeletal muscle cells.

The term "endoplasmic reticulum"(plural - endoplasmic reticula), as used herein, means the intracellular organelle where calcium is stored. The endoplasmic reticulum predominates in heart cells, liver cells, lung cells, pancreas cells, brain cells, muscle cells and osteoblasts.

The term "skeletal muscle relaxants", as used herein, means those compounds which cause relaxation of the skeletal muscle, by acting directly on the muscle or by acting on the neuromuscular junction or by acting on the central nervous system. Dantrolene sodium is an example of a skeletal muscle relaxant that acts directly on the muscle cell by inhibiting calcium release from the sarcoplasmic and endoplasmic reticulum. Baclofen is a centrally acting muscle relaxant which acts by hyperpolarizing gamma-amino butyric acid (GABA) receptors. Succinylcholine acts at the neuromuscular junction by depressing excitatory postsynaptic potentials. Cyclobenzoprine acts on the central nervous system at the brain stem.

The term "septicemia", as used herein, means a blood borne infection which if untreated can lead to sepsis and eventually septic shock.

The term "septic shock", as used herein, means that clinical condition wherein the sepsis has progressed to a state where heart and organ failure are eminent.

The term "trauma", as used herein, means severe physical injury occurring as a result of, but not limited to, automobile accidents, airplane crashes, gun shots, nautical accidents, falls, knife wounds, puncture wounds, and burns.

The term "sympathomimetic agent", as used herein, means a drug which mimics the effects of the sympathetic nervous system. The sympathetic nervous system is stimulated in response to physical or psychological stress. Therefore, agents that mimic the activity of the sympathetic nervous system are useful in the treatment of a variety of clinical disorders, such as shock and heart failure. Thus, a sympathomimetic drug, such as dopamine

exerts a positive inotropic effect on the heart due to stimulation of cardiac β-receptors. Likewise, epinephrine and phenylephrine stimulate the heart through their actions on the cardiac β-receptors. Because a septic patient is physically stressed, and often suffering with a cardiac malfunction, such a patient may be administered a sympathomimetic agents such as dopamine or phenylephrine.

The term "antibody" as used herein means the protein molecule which is formed in response to a stimulus by an antigen, and, is useful in neutralizing the toxic effects of said antigen. There are five (5) major classes of human antibodies, IgG, IgA, IgM, IgD and IgE. Specifically, the bacterial endotoxin is an antigen which stimulates antibody formulation. The antibody formed in response to the endotoxin antigen can be administered to sepsis patients to interfere with the cellular destruction caused by the endotoxin antigen.

## DESCRIPTION OF THE INVENTION

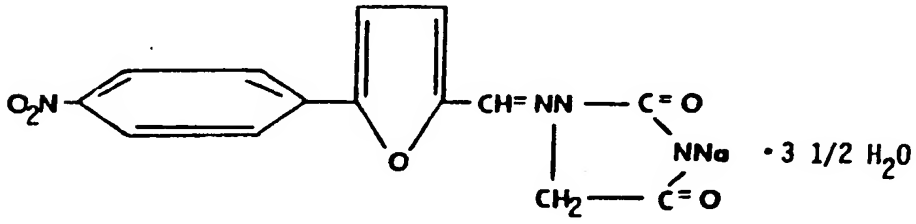
### Active Materials

The active materials useful in the methods of this invention include certain skeletal muscle relaxants, most preferably those which inhibit the release of calcium from the sarcoplasmic and/or endoplasmic reticula and those compounds, not necessarily skeletal muscle relaxants, that inhibit calcium release from the sarcoplasmic and/or endoplasmic reticula.

Certain suitable compounds useful as active materials herein, are described in the following United States Patents, incorporated by reference herein: U.S. Patent 3,415,821 to Davis, et al., issued December 10, 1968; U.S. Patent 3,689,654 to Conklin, et al., issued September 5, 1972; U.S. Patent 4,049,650 to White, issued September 20, 1977; and U.S. Patent 4,822,629 to Pong, issued April 18, 1989. Preferred compounds include dantrolene sodium and azumolene sodium. Most preferred is dantrolene sodium.

Dantrolene Sodium

The methods of this invention comprise the administration of a safe and effective amount of dantrolene sodium to a human or other mammal afflicted with sepsis. Dantrolene sodium (1-[[5-p-nitrophenyl)furfurylidene]-amino]hydantoin sodium salt) has the following structure:



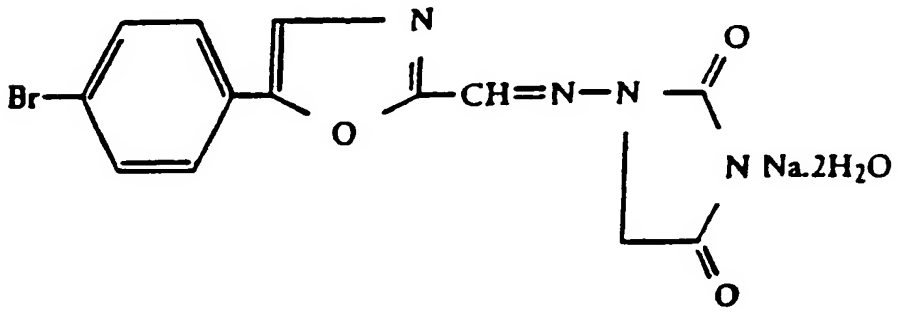

Dantrolene sodium and methods for preparation of dantrolene sodium are described in U.S. Patent 3,415,821 to Davis, et al., issued December 10, 1968, previously incorporated by reference herein. Pharmaceutical compositions comprising dantrolene sodium are described in U.S. Patent 3,689,654 to Conklin, et al., issued September 5, 1972, previously incorporated by reference herein.

Dantrolene sodium is known to those ordinarily skilled in the healing arts as a skeletal muscle relaxant. Dantrolene sodium inhibits the release of calcium from the sarcoplasmic reticulum. *See*, Physicians Desk Reference, 46th Edition (1992). Dantrolene sodium can also be used to treat the symptoms of malignant hyperthermia which include tachycardia, tachypnea, and muscle rigidity. *See*, Physicians Desk Reference, 46th Edition (1992). Additionally, it has been used clinically to treat the symptoms of tetanus which include hyperventilation, muscle rigidity and fever. *See*, Duce, L., and Vigliette, G., "The Use of Dantrolene in A Case of Severe Tetanic Infection", 51(4) *Minerva Anestesiol.* 147-149 (1985); Ortega Cerda, J.J., et al., "Dantrolene Sodium in Tetanus - Report of a Case", 33(1) *Rev. Invest. Clin.* (Mexico) 53-55 (Jan-March 1981) and Rocha, H.,

"Myorelaxant Effect of Dantrolene Sodium in Tetanus", 17 *Rev. Inst. Med. Trop.* (Sao Paulo) 257-262 (July-Aug. 1975).

Azumolene Sodium

The methods of this invention comprise the administration of a safe and effective amount of azumolene sodium to a human or other mammal afflicted with sepsis. Azumolene sodium (1-[[[5-4-bromophenyl-2-oxazolyl]methylene amino]-2,4-imidazolidine-dione,) has the following structure:




Azumolene sodium and methods for preparation of azumolene sodium are described in U.S. Patent 4,049,650 to White issued September 20, 1977, previously incorporated by reference herein. Pharmaceutical compositions comprising azumolene sodium are described in U.S. Patent 4,822,629 to Pong, issued April 18, 1989, previously incorporated by reference herein.

Methods of Treatment

The methods of this invention comprise the administration of a safe and effective amount of a compound that inhibits the release of calcium from the sarcoplasmic and/or endoplasmic reticula, preferably dantrolene sodium or azumolene sodium, to a human or other mammal susceptible to or afflicted with sepsis.

The phrase "safe and effective amount", as used herein, means an amount of a compound or composition large enough to significantly positively modify the symptoms and/or condition to be treated, but small enough to avoid serious side effects (at a reasonable benefit/risk ratio), within the scope of sound medical

judgment. The safe and effective amount of active ingredient for use in the pharmaceutical compositions to be used in the method of the invention herein will vary with the particular condition being treated, the age and physical condition of the patient being treated, the severity of the condition, the duration of the treatment, the nature of concurrent therapy, the particular active ingredient being employed, the particular pharmaceutically-acceptable excipients utilized, and like factors within the knowledge and expertise of the attending physician.

Sepsis is essentially a problem associated with critical care situations. Critical care situations arise when the patient's body is abnormally stressed. Specific critical care situations include, but are not limited to, surgery, trauma, and premature births. Such situations expose the patient to an abnormally high risk of infection. If the infection is unchecked, potentially life threatening sepsis results. Accordingly, sepsis management generally requires rapid intervention. The dantrolene sodium or azumolene sodium may be administered at a level of 0.01 mg/kg to 10.0 mg/kg. The dantrolene sodium or azumolene sodium is preferably administered intravenously to a patient suffering from sepsis at a level of 0.01 mg/kg/hour to 0.50 mg/kg/hour. More preferably, the dantrolene sodium or azumolene sodium is administered at a level of 0.025 mg/kg/hour to 0.35 mg/kg/hour. Most preferably, the dantrolene sodium or azumolene sodium is administered at a level of 0.05 mg/kg/hour to 0.25 mg/kg/hour.

The dantrolene sodium or azumolene sodium may also be administered by any of a variety of known methods of administration, e.g., orally, dermatomucosally (for example, dermally, sublingually, intranasally, and rectally), parenterally (for example, by subcutaneous injection, intramuscular injection, intra-articular injection, intravenous injection), and by inhalation. Thus, specific modes of administration include, but are not limited to, for example, oral, transdermal, mucosal, sublingual, intramuscular, intravenous, intraperitoneal, subcutaneous administration, and topical application.

Dantrolene sodium or azumolene sodium may also be co-administered with antibiotics. The term "co-administered", as used herein, means dosing the subject utilizing all types of adjuvant (i.e. combination) therapies: concurrently (at the same time) and sequentially (one immediately after the other, or one after the other after some rest period). For example, dantrolene sodium or azumolene sodium may be co-administered with certain antibiotic agents, including, but not limited to the penicillins, cephalosporins, ciprofloxacin, erythromycin and the aminoglycosides. Antibiotics known to those skilled in the art, and useful in the methods of this invention, are described in Goodman and Gilman, The Pharmacological Basis of Therapeutics, Chapters 44-49 (8th Edition, 1990). Additionally, the quinolonyl lactam antibiotics may be co-administered with the dantrolene sodium or azumolene sodium. The quinolonyl lactam antibiotics useful for co-administering with dantrolene sodium or azumolene sodium are described in World Patent Publications WO 91/16327 and WO 91/16310, both of Demuth, et al., both published October 31, 1991; both incorporated by reference herein and also in EPO Publications 366,640; 366,193; 366,641; 366,189; 366,643, all of Demuth, et al., all published May 2, 1990; and all incorporated by reference herein.

Additionally, the dantrolene sodium and azumolene sodium may also be co-administered with antibodies to bacterial endotoxins produced by gram-negative bacteria. The bacterial endotoxins produced by the gram-negative bacteria are bacterial membrane lipopolysaccharides. The lipopolysaccharide endotoxin triggers the cascade of events that can lead to sepsis and subsequently to septic shock and death. Specifically, in response to the presence of these lipopolysaccharide endotoxins, mediators of the inflammatory response, including but not limited to, tumor necrosis factor, interleukins, platelet-activating factor, leukotrienes, prostaglandins, interferon, platelets, and bradykinin, are released. The release of these mediators of the inflammatory response causes cellular injury which eventually leads to cellular destruction, and ultimately organ death. *See*,

e.g. Glauser, M. P., et al., "Septic Shock: pathogenesis", 338 *Lancet* 732 (September 21, 1991); Fein, A.M., et al., "Sepsis Syndrome", 10(11) *Infectious Disease Newsletter* 89-96 (1991); Parillo, J.E., "Management of Septic Shock: Present and Future", 115(6) *Ann. Intern. Med.* 491-493 (September 15, 1991); DiPiro, J. T., "Pathophysiology and Treatment of gram-negative sepsis", 47(3) *American Journal of Hospital Pharmacy* S6-S10 (November 1990); Bone, R. C., "The Pathogenesis of Sepsis", 115(6) *Ann. Intern. Med.* 457-469 (September 15, 1991); Dudley, M.N., "Overview of Gram Negative Sepsis", 47(3) *American Journal of Hospital Pharmacy* S3-S6 (November 1990); Barriere, S., et al., "Gram-negative sepsis, the sepsis syndrome, and the role of antiendotoxin monoclonal antibodies", 11(3) *Clin. Pharm.* 223-235 (March 1992) and Murray, M. J., et al., "Sepsis and septic shock - Deadly complications that are on the rise", 90(1) *Postgraduate Medicine* 199-202, 205-6, 208 (July 1991).

Anti-endotoxin antibodies useful in the methods of this invention are described in Barriere, et al., "Therapy Reviews - Gram Negative Sepsis, the sepsis syndrome, the role of antiendotoxin monoclonal antibodies", 11(3), *Clin. Pharm.* 223-225 (March 1992). Specific anti-endotoxin antibodies include, but are not limited to, HA-1A and E5. HA-1A is a monoclonal IgM antibody derived from a human cell line. E5 is a monoclonal IgM antibody derived from a murine cell line. *See*, Ziegler, E.J., et al., "Treatment of gram-negative bacteria and septic shock with HA-1A human monoclonal antibody against endotoxin", 324 *N. Engl. J. Med.* 429-436 (1991) and Greenman, R.L., et al., "A Controlled Clinical Trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram negative sepsis", 266 *JAMA* 1097-1102 (1991). Additionally, antibodies to mediators of the inflammatory response may be co-administered with dantrolene sodium or azumolene sodium. Specifically, antibodies to tumor necrosis factor and the interleukins can be co-administered with the dantrolene or azumolene sodium.

Further, because cardiac function may also be impaired during sepsis, the dantrolene sodium or azumolene sodium may also

be co-administered with a sympathomimetic agent such as, but not limited to, dopamine, epinephrine, or phenylephrine. Sympathomimetic agents known to those skilled in the art, and useful in the methods of this invention, are described in Goodman and Gilman, The Pharmacological Basis of Therapeutics, Chapter 10 (8th Edition, 1990).

The dantrolene sodium or azumolene sodium is administered until the sepsis is resolved. The dantrolene sodium or azumolene sodium may be administered, at a level of 0.01 mg/kg to 10 mg/kg, post-operatively after the sepsis resolves for up to five (5) days to prevent a recurrence of the damaging effects of sepsis. Post-operative administration of dantrolene sodium or azumolene sodium is preferably via intravenous infusion at a level of 0.01 mg/kg/hour to 0.50 mg/kg/hour.

A diagnosis of sepsis may include, but is not limited to, the following symptoms; tachypnea, tachycardia, hypotension, hyperthermia or hypothermia, altered mental state, metabolic acidosis, impaired renal and hepatic function and eventual loss of muscle mass, as determined by elevated blood and urinary nitrogen. Blood cultures are used to determine the presence of infectious organisms. Infectious organisms, known to cause sepsis, include but are not limited to the gram negative bacteria, such as Escherichia coli (E. coli), Klebsiella, Pseudomonas aeruginosa, and Enterobacter; and the gram positive bacteria, such as Staphylococcus epidermidis and Streptococcus faecalis.

Additionally, a number of metabolic and physiologic parameters are examined to diagnose sepsis. Table 1 outlines these parameters.

TABLE 1

Serial Metabolic and Physiologic Parameters to be Examined in Diagnosing Septic Patients

| | | Normal Range |
|---|---|---|
| 1. | Arterial Blood Gases and Plasma Electrolytes | |
| a) | the arterial blood gas analysis includes: | |
| | $PaO_2$ | 90 - 100 mmHg |
| | $PaCO_2$ | 35 - 40 mmHg |
| | pH | 7.35 - 7.42 |
| b) | plasma electrolytes include: | |
| | Na+ | 135 - 140 mEq/lit |
| | K+ | 3.5 - 5.0 mEq/lit |
| | Ca++ | 9.0 - 10.6 mEq/lit |
| | Mg++ | 1.5 - 2.5 mEq/lit |
| | Cl | 95 - 103 mEq/lit |
| | phosphorus, inorganic | 1.8 - 2.6 mEq/lit |
| 2. | Renal Function | |
| a) | 24 hour urine output - variable, minimum urine output 1/2 cc/kg/hr | |
| b) | Serum creatinine | 0.2 - 0.6 mg/dl |
| b) | Serum BUN | 8 - 18 mg/dl |
| c) | Creatinine Clearance | 120 cc/min |
| 3. | Hepatic Function | |
| | plasma enzymes indicative of hepatocellular injury: | |
| a) | SGOT | 8 - 33 u/ml |
| b) | SGPT | 1 - 36 u/ml |
| c) | γ GT | 2 - 39 u/ml |

| | | |
|---|---|---|
| 4. Hematologic and Coagulation System | | |
| a) | hemoglobin | 13 - 17 gm/dl |
| b) | hematocrit | 38 - 45 % |
| c) | white blood cell count | 4,500 - 11,000/ul |
| | neutrophils | 56% |
| | bands | 3% |
| | eosinophils | 2.7% |
| | lymphocytes | 34% |
| | platelet count | 150,000 - 400,000/ul |
| 5. Plasma metabolites | | |
| | glucose | 70 - 110 mg/dl |
| | lactate | < 2.0 mg/dL |

6. Urinary nitrogen balance
   - Variable depending on protein intake.
   - Patient should be in positive nitrogen balance

Values outside the range of the stated normal values are indicative of sepsis. During the intravenous administration of the dantrolene sodium or the azumolene sodium, the parameters of Table 1 are examined on a daily basis to determine whether the sepsis is being resolved.

The methods of this invention also comprise the administration of dantrolene sodium or azumolene sodium pre-operatively to a patient. A patient likely to be treated pre-operatively with dantrolene sodium can be a patient suffering from severe trauma or a patient who will be undergoing major surgery, such as, but not limited to open heart surgery and abdominal surgery, where the risk of infection is enhanced. Such a patient is administered dantrolene sodium or azumolene sodium at a level of 0.01 mg/kg to 10.0 mg/kg. Such a patient is preferably administered dantrolene sodium or azumolene sodium via intravenous infusion pre-operatively, one day prior to surgery, at a level of 0.01 mg/kg/hour to 0.05 mg/kg/hour. Such A patient, who will be undergoing major surgery, can also be

administered dantrolene sodium or azumolene sodium pre-operatively, four hours prior to surgery, in a bolus intravenous dose of 2 mg/kg over a period of twenty minutes.

Administration of dantrolene sodium or azumolene sodium, at a level of 0.01 mg/kg - 10.0 mg/kg, to trauma patients can begin, upon entry into the emergency ward and prior to the surgery to treat the trauma injuries. Preferably, intravenous administration of dantrolene sodium or azumolene sodium, at a level of 0.01 mg/kg/hour - 0.50 mg/kg/hour, can begin upon entry into the Emergency Ward and prior to the surgery to treat the trauma injuries.

## Dosage Forms

The methods of this invention comprise the intravenous administration of dantrolene sodium or azumolene sodium. Dantrolene sodium is commercially available as Dantrium® Intravenous manufactured by Procter & Gamble Pharmaceuticals, Norwich, New York. Dantrium® Intravenous is supplied in 70 ml vials containing 20 mg dantrolene sodium, 3000 mg mannitol and sufficient sodium hydroxide to yield a pH of approximately 9.5 when reconstituted with 60 ml of sterile water for injection USP.

The following non-limiting examples illustrate the methods of this invention.

## Example 1

The patient is a 74 year old white male who is admitted to the hospital because of acute abdominal pain and fever. The patient is taken to the operating room and intra-operative findings reveal a ruptured appendix with spillage of bowel contents and foul-smelling infected peritoneal fluid. After surgical repair, the patient is taken to the surgical intensive care unit (S.I.C.U.). Vital signs on admission to the S.I.C.U. are:

systemic arterial blood pressure - 130/70
pulse - 125 beats per minute (sinus tachycardia)
temperature - 39.5°C rectally
respirations - 10 breaths per minute by mechanical ventilator

Given the diagnosis of intra-abdominal sepsis, the patient is started on broad-spectrum antibiotics and an intravenous infusion of dantrolene sodium (0.025 mg/kg/hour) is begun.

In addition to the patient's vital signs (vide supra) a number of laboratory parameters are followed sequentially to determine the course of the sepsis (see Table 1). The patient's symptoms include high fever, metabolic lactic acidosis, marked loss of muscle mass as determined by elevated blood and urinary nitrogen, deteriorating renal function, and bleeding abnormalities.

In response to the deteriorating condition of the patient, the dantrolene sodium infusion is increased to 0.50 mg/kg/hour. Over the next two to three days, the patient's clinical condition improves as indicated by resolution of the fever, normal acid-base balance, decreased muscle protein breakdown (indicated by the positive nitrogen balance in the 24 hour urine collection), and increased platelet count and decreased bleeding. His dantrolene sodium infusion is continued for seven days until the septic process is resolved. However, the dantrolene sodium is administered for an additional thirty-six hours after the septic process is resolved. He continues to improve and is discharged from the hospital ten days after admission.

Example 2

A 2 kg infant girl is born (one month premature) by cesarean delivery performed for fetal distress. She is cyanotic and respirations are irregular. She is intubated, resuscitated, and placed on mechanical ventilation. Two days later the infant is noted to have increased respiratory rate, decreased blood pressure, and a fall in temperature. Chest x-ray reveals an

infiltrate in the right lower lung lobe. Broad spectrum antibiotics are started and a continuous intravenous infusion of dantrolene sodium is begun at 0.01 mg/kg/hour. Blood cultures taken at two days are positive for Escherichia coli.

A battery of laboratory tests are performed daily to follow the course of the sepsis (see Table 1). The patient's progress is marked by gradual improvement in the respiratory distress and resolution of the septic process. The metabolic acidosis resolves; muscle protein breakdown diminishes (decreased urinary nitrogen excretion); the elevated white blood cell count returns to normal. The infusion of dantrolene sodium is continued for five days until the sepsis is resolved. However, the dantrolene sodium is administered for an additional twenty-four hours after the septic process has resolved. The infant requires two additional weeks of support on a mechanical respirator but eventually is able to be extubated and has a full recovery.

Example 3

The patient is a 45 year old black female who is admitted for evaluation of anemia and blood in the stool. Workup reveals a large bowel carcinoma which is resected at surgery. Three days post-operatively the patient is noted to be febrile (39.8°C), has shaking chills, and is in respiratory distress. She is transferred to the surgical intensive care unit (S.I.C.U.) and workup reveals a leak at the site of the bowel anastomosis. She is taken to the operating room and a diverting colostomy procedure is performed. The abdomen is noted to be grossly infected. She is returned to the S.I.C.U. after surgery and her hospital course is marked by the following:

1. hemodynamic instability - falls in arterial blood pressure
2. high spiking fevers
3. elevated blood glucose concentration
4. metabolic lactic acidosis
5. decreasing renal function - (rising creatinine and decreased urine output)
6. elevated white blood cell count

She is started on broad - spectrum antibiotics. Because of hemodynamic instability and periods of shock, the patient has a pulmonary artery catheter placed which demonstrates decreased cardiac pump function (cardiac output 4.0 liters/min). Stroke volume is only 40 mls with a pulmonary capillary wedge pressure of 22 mm Hg.

The patient is started on dopamine 5 mg/kg/min to increase arterial blood pressure and improve cardiac pump function. A continuous intravenous infusion of dantrolene sodium (0.025 mg/kg/hour) is begun. Thirty minutes after the dantrolene infusion is begun, cardiac output and pump function is improving. Cardiac output has increased from 4.0 to 5.5 liters/min and stroke volume has improved from 40 to 60 ml.

There is continued improvement in the patient's status and she becomes more stable hemodynamically over the next two days. The dantrolene sodium infusion is continued for two days after her fever resolves. Dantrolene sodium is infused for a total of four days. The full spectrum of laboratory tests included in Table 1 are performed on this patient on a daily basis to monitor the course of her sepsis.

## Example 4

The patient is a 24 year old white male, who six months previous had a cadaveric renal transplant for chronic renal failure. He is admitted to the hospital because of high spiking fevers and evidence of a urinary tract infection. Blood and urine cultures are obtained and he is started on broad spectrum

antibiotics. Because of hemodynamic instability and decreasing renal function, a continuous intravenous infusion of dantrolene sodium (0.025 mg/kg/hour) is begun.

The patient is monitored closely in the intensive care unit and serial metabolic and physiologic parameters are followed (see Table 1). Renal function is decreasing as evidenced by a rising serum creatinine and decreasing urine output. The serum creatinine stabilizes at 4.0 mg/dL and urine output is barely adequate at 30 ml/hr. The patient has a steadily improving hospital course and has resolution of his fever and septic picture after four days of the dantrolene sodium infusion.

WHAT IS CLAIMED IS:

1. The use of a compound that inhibits the release of calcium from the sarcoplasmic reticulum or endoplasmic reticulum in the manufacture of a medicament to be used for treating or preventing sepsis in a human or other mammal afflicted with sepsis comprising the administration of a safe and effective amount of said compound that inhibits the release of calcium from the sarcoplasmic reticulum or endoplasmic reticulum.

2. The use of a skeletal muscle relaxant that inhibits the release of calcium from the sarcoplasmic reticulum or endoplasmic reticulum in the manufacture of a medicament for treating or preventing sepsis in a human or other mammal afflicted with sepsis comprising the administration of a safe and effective amount of said skeletal muscle relaxant.

3. The use of dantrolene sodium or azumolene sodium, preferably dantrolene sodium, in the manufacture of a medicament for treating or preventing sepsis in a human or other mammal afflicted with sepsis comprising the administration of a safe and effective amount of said dantrolene sodium or azumolene sodium.

4. The use of a compound, according to Claim 1, wherein the safe and effective amount of said compound is from 0.01 mg/kg to 10.0 mg/kg.

5. The use of a skeletal muscle relaxant, according to Claim 2, wherein the safe and effective amount of said skeletal muscle relaxant is from 0.01 mg/kg to 10.0 mg/kg.

6. The use of dantrolene sodium, according to Claim 3, wherein the safe and effective amount of said dantrolene sodium is from 0.01 mg/kg to 10.0 mg/kg.

7. The use of a compound, according to Claim 1, wherein the safe and effective amount of said compound is from 0.01 mg/kg/hour to 0.50 mg/kg/hour.

8. The use of dantrolene sodium, according to Claim 3, wherein the safe and effective amount of dantrolene sodium is from 0.01 mg/kg/hour to 0.50 mg/kg/hour.

9. The use of a compound, according to Claim 1, wherein administration of said compound is concurrent with antiobiotics and is via intravenous infusion.

10. The use of dantrolene sodium, according to Claim 3, wherein administration of said dantrolene sodium is concurrent with antibiotics and is via intravenous infusion.

# INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 93/07698

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/415 A61K31/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | PROC. NATL. ACAD. SCI. USA<br>vol. 90, no. 9 , 1 May 1993<br>pages 3933 - 3937<br>S.-K. SONG ET AL. 'Increased intracellular Ca2+: A critical link in the pathophysiology of sepsis'<br>see the whole document | 1-3 |
| Y | --- | 4-10 |
| Y | CRIT. CARE MED.<br>vol. 20, no. 4 , April 1992<br>page S48<br>J.C. TODD ET AL. 'Effect of sepsis on erythrocyte intracellular calcium homeostasis'<br>see abstract | 1-10 |
| | ---<br>-/-- | |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 October 1993 | |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+ 31-70) 340-3016 | KRAUTBAUER, B |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Int. nal Application No
PCT/US 93/07698

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | INFECT. IMMUN.<br>vol. 59, no. 5 , 1991<br>pages 1599 - 1604<br>T.J. BALDWIN ET AL. 'Elevation of intracellular free calcium levels in HEp-2 cells infected with enteropathogenic Escherichia coli'<br>see the whole document | 1-10 |
| Y | ANAESTH. ANALG.<br>vol. 73, no. 3 , 1991<br>pages 289 - 294<br>D.S. BEEBE ET AL. 'Is dantrolene safe to administer in sepsis? The effect of dantrolene after endotoxin administration in dogs and rats'<br>cited in the application<br>see the whole document | 1-10 |
| Y | CIRCULATORY SHOCK<br>vol. 19, no. 1 , 1986<br>pages 69 - 74<br>S. BOSSON ET AL. 'Increased survival with calcium antagonists in antibiotic-treated bacteremia'<br>cited in the application<br>see the whole document | 1-10 |
| Y | CIRC. SHOCK<br>vol. 37, no. 3 , July 1992<br>pages 226 - 229<br>G.P. ZALOGA ET AL 'Low dose calcium administration increases morality during septic peritonitis in rats'<br>see the whole document | 1-10 |
| Y | ARCH. SURG.<br>vol. 125, no. 2 , 1990<br>pages 274 - 275<br>R.C. BOORSE ET AL. 'Neuroleptic malignant syndrome in a multiple trauma patient'<br>see the whole document | 1-10 |
| Y | US,A,4 861 790 (R.L. WHITE ET AL.) 29 August 1989<br>see the whole document | 1-10 |
| Y | ANESTHESIOLOGY<br>vol. 76, no. 5 , May 1992<br>pages 711 - 719<br>J.R. LOPEZ 'Effects of dantrolene on myoplasmic free (ca2+) measured in vivo in patients susceptible to malignant hyperthermia'<br>see the whole document | 1-10 |

1

# INTERNATIONAL SEARCH REPORT

rnational application No.

PCT/US93/07698

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims searched completely: 3,6,8
Claims searched incompletely: 1,2,4,5,7,9,10
Compounds are not well defined by their pharmacological activity. The search had therefor to be restricted to the compounds explicitely...(see annex)

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

...mentioned in the claims and the general inventive concept. A general expression like "antibiotics" does not make sufficiently clear which compounds are meant. The search had to be restricted to the general inventive concept here.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. nal Application No
PCT/US 93/07698

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US-A-4861790 | 29-08-89 | NONE | |